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TITLE: **METHODS AND COMPOSITIONS FOR HEAT ACTIVATED GENE THERAPY USING CYTOLETHAL DISTENDING TOXIN**

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METHODS AND COMPOSITIONS FOR HEAT ACTIVATED GENE THERAPY USING CYTOLETHAL DISTENDING TOXIN

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 60/442,473 filed on January 24, 2003, entitled "Methods and Compositions for Heat Activated Gene Therapy Using Cytolethal Distending Toxin."

FIELD OF THE INVENTION

[0002] The present invention relates to methods and compositions for conducting gene therapy. More particularly, the present invention relates to cytolethal gene therapy using toxins.

BACKGROUND OF THE INVENTION

[0003] Gene therapy holds great promise as a clinical treatment for a variety of human maladies. Gene therapy involves the delivery of an exogenous gene or other polynucleotide to a cell or plurality of cells. The exogenous gene carries the therapy in the sense that it is a composition that will, by way of its introduction into the cell, confer benefits onto the cell and/or host. The benefits conferred onto the cell are typically designed to be therapeutic to the host. Thus, the benefits are typically designed to correct a deficiency or to kill an undesirable cell.

[0004] Many different therapeutic strategies for gene therapy have been devised. In one approach, a natural gene that is defective for one reason or another is replaced with an exogenous copy of the gene that is more suitable for achieving the function and/or purpose of the gene. For example, a wild-type exongenous gene can be introduced as a replacement for a mutant, less effective natural gene. This

approach holds promise for disease conditions in which an individual produces ineffective gene products, such as cystic fibrosis.

[0005] In another approach, a special gene, frequently referred to as a "suicide gene", is delivered to one or more cells of interest. The suicide gene encodes a gene product that is toxic to the cell. Accordingly, production of the suicide gene product ultimately leads to the death of the cell. This approach holds promise for treatment of disease conditions in which it is desirable to eliminate certain cells from a host, such as in various forms of cancer.

[0006] In both of these approaches, delivery of the exogenous gene to the cells of interest presents a challenge. Frequently, a vector of some type is used to deliver the gene to the cells or tissue being treated. For several reasons, viral vectors are currently the most frequently used vector in gene therapy procedures. The natural replication cycle of a virus enables the vector to reproduce its genetic contents, including any exogenous genes, using the molecular machinery of an infected cell. Subsequently, the infected cell releases the resultant daughter vectors to the surrounding environment. This allows the exogenous gene to be repeatedly introduced into new cells, thereby expanding the area in which the therapy occurs beyond the originally infected cell.

[0007] Some benefits of using a viral vector are lost, however, when a suicide gene is utilized. By nature, the suicide gene encodes a product that is toxic to the cell. Thus, the gene encodes a product that will ultimately kill the cell. If the suicide gene is sufficiently toxic, the cell may perish before the vector is able to replicate and repackage itself for delivery to other cells. As a result, the distribution of the therapy,

i.e., the suicide gene, can be stopped prior to expansion beyond the original cells, which may decrease the effectiveness of the therapy.

[0008] Some toxins, such as the shiga, cholera, and diptheria toxins, appear to be sufficiently toxic to create this situation. Indeed, elaborate molecular "choke" mechanisms have been used to slow the production of the suicide gene product in order to allow production and packaging of viral components.

BRIEF SUMMARY OF THE INVENTION

[0009] In one aspect, the present invention provides gene therapy vectors that include one or more suicide gene and various control elements. The suicide gene is a gene for a cytolethal distending toxin (CDT), and is preferably a gene for a B subunit of a CDT. The control elements include an inducible promoter and an antisense oligonucleotide that inhibits expression of a nucleic acid that encodes a DNA repair protein. The inducible promoter is preferably a heat shock promoter, and particularly preferably a segment of a heat shock promoter that is strictly inducible by heat shock. The antisense oligonucleotide preferably inhibits expression of a DNA repair protein that functions to repair DNA damaged in a manner that reflects the type of damage induced by the suicide gene. Preferably, the antisense oligonucleotide inhibits expression of ku70, a protein that is critical to the functioning of the non-homologous end joining DNA repair mechanism. This inhibits repair of double-strand breaks in DNA, such as those induced by B subunits of CDTs.

[0010] The control elements ensure that the suicide gene is expressed only under certain conditions. This, in turn, provides an opportunity for the vector to replicate and repackage for delivery to additional cells prior to expression of the

suicide gene. Also, the control elements prevent the cell from repairing damage induced by the suicide gene. Interestingly, the ku70 antisense control element also sensitizes the cell to other forms of DNA-damaging therapy, such as radio- and chemotherapy.

[0011] Thus, a preferred embodiment of a composition according to the present invention comprises a vector including a polynucleotide that has a first nucleotide sequence that encodes a B subunit of a CDT, second nucleotide that encodes an antisense oligonucleotide that inhibits expression of ku70, and a heat shock promoter that is strictly inducible by heat and that is positioned to promote expression of the first and second nucleotide sequences.

[0012] In another aspect, the present invention also provides methods of conducting gene therapy. The methods utilize a vector according to the present invention. Accordingly, the methods include steps directed at inducing the inducible promoter to drive expression of the suicide gene and the antisense oligonucleotide. As indicated above, the preferred embodiment of the vector according to the present invention includes a heat shock promoter as one of the control elements.

[0013] Appropriate steps for inducing the promoter, therefore, include elevating the temperature of the cell and/or tissue to which the vector has been delivered to a temperature above normal body temperature. Various hyperthermia techniques can be employed to accomplish this temperature elevation. Thus, a preferred method according to the present invention comprises delivering a vector according to the present invention to a tumor. Also, the method includes elevating the temperature of the tumor to a temperature that is sufficient to induce the promoter to express the suicide gene and the antisense oligonucleotide.

DETAILED DESCRIPTION OF THE INVENTION

[0014] The present invention provides methods and compositions for conducting gene therapy. The methods and compositions utilize cytolethal distending toxins, or subunits thereof. While the following description of preferred embodiments and methods provides examples of the present invention, the description is not intended to limit the scope of the invention in any manner. Rather, the description serves to enable a person of ordinary skill in the relevant art to practice the present invention.

[0015] 1. Definitions

[0016] The following definitions apply for the indicated terms:

[0017] The term "gene therapy" is given its ordinary meaning in the art. Briefly, "gene therapy" refers to the transfer of genetic material (e.g., a DNA or RNA polynucleotide) of interest into a host cell and/or tissue to treat or prevent a disease condition. The genetic material of interest typically encodes a product whose in vivo production is desired. The genetic material of interest can also include various control elements, such as transcriptional promoters. In the present invention, the genetic material of interest includes a cytolethal distending toxin, or a subunit thereof, an antisense oligonucleotide, and an inducible promoter.

[0018] As used herein, the term "cytolethal distending toxin" (CDT) refers to a family of multisubunit toxins produced by a variety of bacteria. Each CDT is capable of inducing cell cycle arrest at G2/M in a variety of cell types, including Chinese Hamster Ovary (CHO), Hela, Hep-2, Vero, CaCo-2, human keratinocyte cell line (HaCat), hamster lung (Don) fibroblast and human T lymphocyte cells. (Johnson, W. and Lior, H., 1988, Micorbial Pathogenesis, "A new heat labile cytolethal distending

toxin (CLDT) produced by Campylobacter spp., " Vol 4: pp. 115-126; Pickett, C. and Whitehouse, C., 1999, Trends Microbiol, "The cytolethal distending toxin family," Vol. 7(7): pp. 292-7). The cell cycle arrest results in a cessation of cell division. The CDTs are also capable of producing other effects, such as progressive cellular distention, in some of these cell types. Further, at least two cell types, Y-1 adrenal and NIH 3T3 fibroblast cells, are not killed in response to CDTs (Elwell, C. and Dreyfus, L., 2000, Molecular Microbiology, "DNase I homologous residues in CdtB are critical for cytolethal distending toxin-mediated cell cycle arrest," Vol 37(4): pp. 952-963).

[0019] A complete CDT represents a multiunit assembly of gene products from at least three genes: cdtA, cdtB, and cdtC. The genes for the CDTs have been cloned and characterized from a variety of bacterial species, including Haemophilus ducreyi; Cimpylobacter jejuni, and Escherichia coli, (see below).

[0020] As used herein, the term "B subunit", as it relates to a specific subunit of the multiunit CDT, refers to the product of the cdtB gene.

[0021] As used herein, the term "antisense oligonucleotide" refers to an oligonucleotide that has at least a partially complementary sequence to another oligonucleotide (the "sense oligonucleotide") such that the antisense is able to hybridize to the sense oligonucleotide.

[0022] As used herein, the term "inducible promoter" refers to a transcriptional promoter that promotes transcription of appropriate genes when certain environmental conditions are present.

[0023] As used herein, the term "heat shock promoter" refers to an inducible promoter that promotes transcription of appropriate genes when one ore more of a

variety of stressful environmental conditions are present, including elevated temperatures (i.e., heat shock) and oxidative stress.

[0024] As used herein, the term "segment of a heat shock promoter" refers to a portion of a whole heat shock promoter.

[0025] As used herein, the term "strictly inducible by heat shock" refers to an ability of a heat shock promoter or segment thereof to promote transcription of appropriate genes at high level under heat shock conditions, while substantially not promoting higher transcription levels under other stressful, non-temperature related conditions, such as oxidative stress.

[0026] As used herein, the term "non-homologous end-joining DNA repair mechanism" refers to the DNA repair mechanism that operates to repair double-strand breaks in DNA in eukaryotic cells by blunt end ligation. The enzyme complex that accomplishes this repair mechanism includes the proteins ku70 and ku80, the DNA-dependent protein kinase (DNA-Pkes), and the DNA ligase IV, which functions in conjunction with the protein XRCC4. The ku70 and ku80 proteins form a dimer that binds the DNA double-strand end, and the repair mechanism operates by ligating the blunt ends created by the double strand break.

[0027] As used herein, the term "hybridization" refers to cumulative hydrogen bonding between complimentary nucleoside or nucleotide bases in a pair of oligonucleotides. The cumulative bonding, when sufficient, bonds the oligonucleotides to each other.

[0028] As used herein, the term "complimentary" refers to the ability of a pair of nucleoside or nucleotide bases to specifically bond with eachother through hydrogen bonding. For example, in DNA, Adenine (A) and Thymidine (T) are

complementary bases, and Cytosine (C) and Guanine (G) are complementary bases. The same is true in RNA, except that Uracil (U) is complimentary to Adenine (A).

[0029] As used herein, the term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA), or mimetics thereof. The term encompasses oligers and polymers that include naturally occurring bases, non-naturally occurring bases that function similar to natural bases, and combinations thereof.

[0030] As used herein, the term "polynucleotide" refers to an oligomer or polymer of RNA or DNA in the same manner as an "oligonucleotide". The difference between the two terms is merely one of relative size: a polynucleotide refers to a larger entity, which may contain one or more oligonucleotides.

[0031] 2. Description of Preferred Embodiments and Methods.

[0032] In one aspect, the invention provides a construct for use in cytolethal gene therapy procedures. The construct is preferably contained within a vector, and preferably includes a first polynucleotide having a nucleotide sequence that encodes a gene for a B subunit of a CDT, a second polynucleotide having a nucleotide sequence that encodes an antisense oligonucleotide that inhibits expression of a nucleic acid encoding a DNA repair protein, and an inducible promoter operably linked to the first and second polynucleotides.

[0033] Essentially any agent that can contain the constructs according to the present invention can be used as the vector. Indeed, a wide variety of agents are recognized as being suitable for use in gene therapy procedures, and any of these suitable vectors can be utilized in the present invention. United States Patent No.

6,093,567 to Gregory, et al., for GENE THERAPY FOR CYSTIC FIBROSIS provides a detailed discussion of some suitable vectors. Examples of known vectors that are suitable for use in the present invention include polymeric molecules, genetic cassettes, plasmids, phages, viruses, and pseudoviruses. Viruses are currently the most frequently used vectors in gene therapy, and several types of virus vectors can be used in the present invention. Examples of suitable virus vectors include papoviruses, lentiviruses, adenoviruses, adeno-associated viruses, vaccina viruses, herpes viruses, and retroviruses.

[0034] The following United States Patents provide descriptions of several types of viral vectors: 6,140,111 to Riviere, et al. for RETROVIRAL GENE THERAPY VECTORS AND THERAPEUTIC METHODS BASED THEREON; 6,106,826 to Brandt, et al. for REPLICATION COMPETENT, AVIRULENT HERPES SIMPLEX VIRUS AS A VECTOR FOR NEURAL OCCULAR GENE THERAPY; and 6,140,087 to Graham, et al. for ADENOVIRUS VECTORS FOR GENE THERAPY.

[0035] Preferably, the vector comprises a viral vector. Particularly preferably, the vector comprises an adenovirus vector.

[0036] It should be noted that the construct of the present invention itself can be considered a vector in accordance with the present invention. That is, the construct comprising the gene encoding the CDT B subunit gene, the antisense oligonucleotide, and the inducible promoter operably linked to these elements can, by itself, comprise the vector of the present invention. Naked DNA can be used in gene therapy procedures to deliver the gene of interest to a particular cell. This approach may be desired if the advantages of using other vector types, such as viral vectors, are not desired. For example, the inventor has discovered that the B

subunit produces toxicity not only in the original cell, but also in some of the immediately surrounding cells. As a result, the need for a viral vector and its ability to replicate, repackage daughter vectors, and deliver the construct to the surrounding cells may be eliminated. In this case, use of the construct itself, i.e., naked DNA, as the vector may be suitable.

[0037] The first polynucleotide has a nucleotide sequence that encodes a gene for a B subunit of a CDT. The B subunit of CDTs displays enzymatic activity similar to that of DNAse I. (Lara-Tejero M. and Galan, J.; 2000, Science, "A Bacterial Toxin That Controls Cell Cycle Progression as a Deoxyribonuclease I - Like Protein", Vol 290: pp. 354-357; Elwell, C. and Dreyfus, L., 2000, Molecular Microbiology, "DNase I homologous residues in CdtB are critical for cytolethal distending toxin-mediated cell cycle arrest," Vol 37(4): pp. 952-963). That is, the β subunit has endonuclease activity, and can cause disruption of chromatin structures (Lara-Tejero M. and Galan, J.; 2000, Science, "A Bacterial Toxin That Controls Cell Cycle Progression as a Deoxyribonuclease I - Like Protein", Vol 290: pp. 354-357). This DNAse I activity may be responsible for the ability of the CDT to arrest the cell cycle (Elwell, C. and Dreyfus, L., 2000, Molecular Microbiology, "DNase I homologous residues in CdtB are critical for cytolethal distending toxin-mediated cell cycle arrest," Vol 37(4): pp. 952-963).

[0038] The B subunit of a CDT is encoded by the cdtB gene. The cdtB gene has been cloned and characterized from a variety of organisms, including Haemophilus ducreyi (Cope, L., Lumbley, S., Latimer, J., Klesney-Tait, J., Stevens, M., Johnson, L., Purven, M., Munson, R., Lagergard, T., Radolf, J. and Hansen, E., 1997, Proc. Natl. Acad. Sci. USA, "A diffusible cytotoxin of Haemophilus ducreyi,"

Vol 94: pp. 4056-4061) (SEQ ID 1), Campylobacter jejuni (Pickett, C., Pesci, E., Cottle, D., Russell, G., Erdem, A. and Zeytin, H., 1996, Infection and Immunity, "Prevalence of Cytolethal Distending Toxin Production in Campylobacter jejuni and Relatedness of Campylobacter sp. cdtB Genes," pp. 2070-2078) (SEQ ID 2), and Escherichia coli (Scott, D., and Kaper, J., 1994, Infection and Immunity, "Cloning and Sequencing of the Genes Encoding Escherichia coli Cytolethal Distending Toxin," pp. 244-251; (SEQ ID 3) Pickett, C., Cottle, D., Pesci, E., and Bikah, G., 1994, Infection and Immunity, Cloning, Sequencing, and Expression of the Escherichia coli Cytolethal Distending Toxin Genes," pp. 1046-1051) (SEQ ID 4). The sequencing information for these various cdtB genes reveal that considerable heterogeneity among cdtB (and cdtA and cdtC, see below) genes may exist (Pickett, C., Pesci, E., Cottle, D., Russell, G., Erdem, A. and Zeytin, H., 1996, Infection and Immunity, "Prevalence of Cytolethal Distending Toxin Production in Campylobacter jejuni and Relatedness of Campylobacter sp. cdtB Genes," pp. 2070-2078). Indeed, the predicted amino acid sequences for the proteins encoded by the cdtB genes listed as SEQ ID 3 and SEQ ID 4 share only 55-56% identity, despite being from different strains of the same bacteria (Pickett, C., Pesci, E., Cottle, D., Russell, G., Erdem, A. and Zeytin, H., 1996, Infection and Immunity, "Prevalence of Cytolethal Distending Toxin Production in Campylobacter jejuni and Relatedness of Campylobacter sp. cdtB Genes," pp. 2070-2078, analyzing sequences presented in Scott, D., and Kaper, J., 1994, Infection and Immunity, "Cloning and Sequencing of the Genes Encoding Escherichia coli Cytolethal Distending Toxin," pp. 244-251 and Pickett, C., Cottle, D., Pesci, E., and Bikah, G., 1994, Infection and Immunity,

Cloning, Sequencing, and Expression of the Escherichia coli Cytolethal Distending Toxin Genes," pp. 1046-1051).

[0039] Any cdtB gene can be used in the first polynucleotide of the construct of the present invention, so long as the gene product possesses the desired toxicity. The B subunit represents the product of the suicide gene of the construct. Accordingly, any cdtB gene can be utilized so long as is encodes a gene product that possesses the toxicity associated with the B subunit protein, as discussed above. Preferably, the cdtB gene encodes a gene product that possesses the DNAse I-like activity associated with the B subunit. Also preferable, the cdtB gene encodes a gene product that is able to induce cell cycle arrest at G2/M in certain cells, such as CHO, HeLa, Hep-2, Vero, CaCo-2, HaCat, hamster lung fibroblast, and human T-lymphocyte cells (see, e.g., Johnson, W. and Lior, H., 1988, Micorbial Pathogenesis, "A new heat labile cytolethal distending toxin (CLDT) produced by Campylobacter spp., " Vol 4: pp. 115-126; and Pickett, C. and Whitehouse, C., 1999, Trends Microbiol, "The cytolethal distending toxin family," Vol. 7(7): pp. 292-7).

[0040] Preferably, the cdtB gene has the nucleotide sequence listed as SEQ ID 5. This sequence represents a cdtB gene cloned and characterized from Escherichia coli strain MBU. E 412 (Genbank accession number AF373206).

[0041] It should be noted that, while the CDTs are multiple subunit entities, the use of the other subunits, i.e., cdtA and cdtC, is not desirable in the present invention because these subunits appear to facilitate the entry of the B subunit into cells. If these subunits were included, widespread dispersion of the B subunit might be achieved, even though not intended. The distribution of the B subunit in the cells or tissue surrounding the cell in which the B subunit was originally introduced is, at

this point, advantageously limited to those which the B subunit enters via the vector or on its own.

[0042] The second polynucleotide includes a nucleotide sequence that encodes an antisense oligonucleotide. Antisense oligonucleotides specifically hybridize with another oligonucleotide, the target or sense oligonucleotide, to interfere with the normal functioning of the target oligonucleotide. For example, if the target oligonucleotide is RNA, the functions to be interfered with include translocation of the RNA to the site of translation, translation of protein from the RNA, splicing of the RNA to yield one or more RNA species, and catalytic activity which may be engaged or facilitated by the RNA.

[0043] The use of antisense oligonucleotides is becoming more frequent and accepted in the areas of research and diagnostics. For example, antisense oligonucleotides that inhibit specific gene expression or protein translation are frequently used by those of ordinary skill in the art to elucidate the function of particular genes and/or protein. Further, antisense oligonucleotides are commonly used to distinguish between various functions of various members of a particular biological pathway. Also, antisense oligonucleotides are used to study the relationship between seemingly unrelated biological entities.

[0044] Those of ordinary skill in the art have also harnessed the use of antisense oligonucleotides as therapeutic agents. Antisense oligonucleotides have been proposed and used as therapeutics in a variety of disease conditions in animal and man. Indeed, antisense oligonucleotides have been administered to humans in a variety of clinical trials in a safe and effective manner.

[0045] United States Patents 6,287,860 to Monia, et al. for ANTISENSE INHIBITION OF MEKK2 EXPRESSION and 6,251,873 to Furusako, et al. for ANTISENSE COMPOUNDS TO CD14 provide additional background information on antisense oligonucleotides and their use in research and medicine.

[0046] The second polynucleotide includes a nucleotide sequence that encodes an antisense oligonucleotide. The antisense oligonucleotide operates to inhibit the function of a target nucleic acid that is involved in a DNA repair mechanism. The CDT B subunit encoded by the first polynucleotide induces DNA damage in the host cell, and the antisense oligonucleotide operates to prevent the cell from utilizing its natural DNA repair mechanism(s) to repair the damage. This helps to ensure that the B subunit toxin will ultimately succeed in lysing the host cell.

[0047] Eukaryotic cells have a variety of DNA repair mechanisms that operate to repair damaged DNA.

[0048] As discussed above, the B subunit of CDTs possesses DNAse I-like activity, which presumably allows the toxin to arrest the cell cycle (see above; see also Lara-Tejero M. and Galan, J.; 2000, Science, "A Bacterial Toxin That Controls Cell Cycle Progression as a Deoxyribonuclease I - Like Protein", Vol 290: pp. 354-357.; and Elwell, C. and Dreyfus, L., 2000, Molecular Microbiology, "DNase I homologous residues in CdtB are critical for cytolethal distending toxin-mediated cell cycle arrest," Vol 37(4): pp. 952-963). Thus, the B subunit has endonuclease activity that results in double strand breaks and blunt ends. Accordingly, the antisense oligonucleotide preferably interferes with the normal functioning of a sense oligonucleotide involved in a DNA repair mechanism that is able to repair these double-stranded breaks.

[0049] The non-homolgous end-joining mechanism is a DNA repair mechanism in eukaryotic cells that operates to repair double-strand breaks by blunt end ligation. The mechanism includes a series of proteins that accomplish the ligation. Specifically, the mechanism utilizes the ku70 and ku80 proteins, the DNA-dependent protein kinase (DNA-Pkes), and the DNA ligase IV, which functions in conjunction with the protein XRCC4. The antisense oligonucleotide can be any antisense that hybridizes to a target oligonucleotide in a manner that interferes with the functioning of the non-homolgous end-joining DNA repair mechanism. For example, the gene for the ku80 protein has been cloned.

[0050] Preferably, the antisense oligonucleotide interferes with the normal functioning of a target oligonucleotide that encodes a gene for the ku70 protein.

[0051] The cDNA encoding ku70 has been published.

[0052] The antisense oligonucleotide can be designed to interfere with the normal functioning of the appropriate gene (e.g., the gene encoding the ku70 protein) or an mRNA molecule transcribed from the gene. Because the ku70 participates in the activity of interest for the present invention (i.e., repair of DNA double-strand breaks) and it is not believed that the mRNA has any such participation, the antisense oligonucleotide preferably interferes with the normal functioning of the mRNA for the ku70 protein. This, it is preferred that the antisense oligonucleotide interferes with the translation of the ku70 protein from the mRNA.

[0053] It is known in the art that an antisense oligonucleotide need not hybridize to the entire length of the target nucleotide to achieve the desired interference with functionality. Indeed, it may be possible to determine a specific site or sites within the target oligonucleotide that can be targeted such that the desired

interference occurs. Such sites can be determined by gene walk experiments known to those skilled in the art. In these experiments, a series of overlapping antisense oligonucleotides are evaluated for their ability to achieve the desired interference.

[0054] However, interference can be assured by the use of an antisense oligonucleotide that is a "full length" antisense. That is, the full length antisense is able to hybridize to the entire target oligonucleotide (e.g., the gene or mRNA). Because of this full length hybridization, the desired interference is assured to occur. Thus, in the present invention, the antisense oligonucleotide preferably is a full length antisense for the ku70 mRNA. The full length antisense, therefore, preferably is able to hybridize to the mRNA from at least the translation initiation codon (5'-AUG) (also referred to as the "start codon") to the translation termination codon (also referred to as the stop codon). The step codon in mRNA can be one of three sequences: 5'-UAA, 5'-UAG, and 5'UGA. It should be noted that some genes have alternative translation initiation codons having the following sequences: 5'GUG, 5'UUG, or 5'CUG. Further, the following sequences have been shown to function as start codons in vivo: 5' AUA, 5' ACG and 5'-CUG.

[0055] It is also known in the art that an mRNA may have several alternative translation initiation codons, any one of which may be preferentially used in a particular cell or tissue, or under a particular set of conditions. To the extent such multiple start codons are present in the target oligonucleotide, it is preferred that the antisense oligonucleotide be able to hybridize to all start codons present. This ensures the desired interference with the mRNA function.

[0056] It will be appreciated by those of skill in the art that an antisense oligonucleotide need not be 100% complimentary to its target oligonucleotide to achieve hybridization that accomplishes the desired interference. Indeed, only a sufficient degree of complimentarity is required. The sufficient degree of complimentarity required is that degree which avoids non-specific binding to non-target oligonucleotides while allowing enough hybridization to the target oligonucleotide to achieve the desired interference with the function of the target oligonucleotide.

[0057] Considering the above, a particularly preferred antisense oligonucleotide has a sequence complimentary to the sequence listed as SEQ ID 6.

[0058] The construct of the present invention includes an inducible promoter operably linked to the first and second polynucleotides. That is, the construct includes an inducible promoter that is able to initiate the transcription of the B subunit gene and the antisense oligonucleotide.

[0059] Inducible promoters are those promoters able to induce transcription of appropriate genes when certain environmental conditions are met. A variety of inducible promoters, which correspond to a variety of environmental conditions that induce the promoters, are known to those skilled in the art. Examples include the arabinose promoter, the metallothioneine promoter, and the heat shock promoters. The use of an inducible promoter is desired because it is desirable to regulate the expression of the B subunit and antisense by environmental conditions, such as temperature (see below). Accordingly, any suitable inducible promoter can be utilized. The choice of inducible promoter should be made to ensure that the B subunit and the antisense oligonucleotide are not constitutively expressed. Also, the

inducible promoter should promote the expression of these elements under only the environmental conditions that will be used to regulate the gene therapy procedure. The heat shock promoters provide suitable inducible promoters for use in the present invention. As indicated above, these promoters induce transcription when certain stressful environmental conditions are present, such as elevated temperatures and oxidative stress.

[0060] The human Hsp70B heat shock promoter is suitable for use in the present invention. The sequence for this promoter is listed in SEQ ID 7.

[0061] Preferably, the inducible promoter is strictly inducible by heat shock. This characteristic ensures that activation of transcription of the B subunit and antisense oligonucleotide will occur only in the presence of heat shock, which allows for tight regulation of the gene therapy procedure. This strict inducibility can be accomplished by using a segment of a heat shock promoter. Indeed, segments of the human Hsp70B heat shock promoter that are strictly inducible by heat shock have been determined (Schiller, P., Amin, J., Ananthan, J, Brown, M., Scott, W., and Voellmy, R., 1988, J. Mol. Biol., "Cis-acting Elements Involved in the Regulated Expression of a Human HSP70 Gene, Vol. 203: pp.97-105; Voellmy, R., Ahmed, A., Schiller, P., Bromley, P., and Rungger, D., 1985, Proc. Natl. Acad. Sci. USA, "Isolation and functional analysis of a human 70,000-dalton heat shock protein gene segment," Vol. 82: pp. 4949-4953). A sequence of a preferred such segment appears as SEQ ID 9. Molecular vectors utilizing such a segment are readily available from commercial sources, and include the p2500-CAT and pD35X vectors available from Stressgen Biotechnologies Corporation of Victoria, British Columbia, Canada.

[0062] It will be appreciated by those skilled in the art that the construct of the present invention can be made according to standard molecular biology and genetic engineering techniques. Such techniques include using expression vectors, digesting an expression vector with a restriction endonuclease enzyme, and isolating a desired vector product. Also, packaging a construct of the present invention into a vector such as a viral vector, if desired, can be accomplished according to techniques known to those skilled in the art.

[0063] The present invention also provides methods of conducting cytolethal gene therapy. The methods according to this invention utilize vectors according to the invention, and comprise delivering the vector to a desired cell and placing the cell under environmental conditions appropriate to induce the promoter. As a result, the B subunit and antisense oligonucleotide are expressed. The B subunit causes DNA damage in the cell, and the natural DNA repair mechanism of the cell is hindered by the antisense oligonucleotide. Ultimately, this results in the death of the cell.

[0064] The vector used in the methods of the invention can be any vector in accordance with the invention, as described above. Thus, viral vectors as well as plasmids and naked DNA vectors, in addition to other suitable vectors, can be utilized. Preferably, a vector according to the preferred embodiment of the invention is used. That is, it is preferred to utilize an adenoviral vector containing a polynucleotide encoding a cdtB gene, an antisense oligonucleotide that inhibits the expression of ku70, and a segment of a heat shock promoter that is strictly inducible by heat shock and that is positioned to promote expression of the cdtB gene and the antisense oligonucleotide.

[0065] The cell can comprise any eukaryotic cell in which the vector can achieve its function. Thus, the cell should contain DNA and should have a natural DNA repair mechanism. Essentially any eukaryotic cell, therefore, can be used in the procedure.

[0066] As a controllable cytolethal procedure, the methods of the present invention provide techniques suitable for killing one or more cells of interest. As such, the methods are well-suited for eliminating undesired cells from a tissue or host. An example of such a type of cell is a cancerous cell, which may be contained in a solid tumor. The methods can be used on a variety of solid tumors, including colon, prostate, breast, lung, skin, liver, bone, pancreas, ovary, testes, bladder, kidney, brain, nerve, and head and neck tumors.

[0067] Accordingly, the cell is preferably a cancerous cell contained within a solid tumor.

[0068] Delivering the vector to the desired cell or cells can be accomplished by any suitable technique. For example, systemic delivery routes such as intravenous injection can be utilized if the vector is able to specifically enter the desired cell. Preferably, however, the vector is directly delivered to the cell or tissue of interest. This direct delivery is preferably accomplished by a direct injection technique. Direct injection can be accomplished using a syringe, needle, or other delivery device suitable for delivering other therapeutic agents to tissue. When the cell of interest is in a solid tumor, the vector can be injected with a standard syringe into the tissue mass. Preferably, using this technique, the syringe is inserted into the tissue at a depth and position that ensures delivery of the vector to the approximate geometric center of the tissue mass. Alternatively, the position at which the vector is

delivered can be varied according to the needs of the procedure being conducted. Also, microinjection techniques can be utilized to directly place the vector inside of the cell of interest.

[0069] The quantity and concentration of vectors delivered to a cell and/or tissue of interest can be optimized based on a number of parameters, including the ability of the vector to replicate and the number of cells in which treatment is desired. Preferably, when an adenoviral vector is used with a solid tumor, a multiplicity of infection of between approximately 1 and 100 is used.

[0070] As indicated above, the methods of the present invention also include placing the desired cell under environmental conditions appropriate to induce the inducible promoter. Thus, the details of this step will depend on the nature of the inducible promoter, and may include adding a particular compound to the cellular environment or inducing a particular type of environmental stress.

[0071] When a vector according to the preferred embodiment is utilized, i.e., the inducible promoter is a heat shock promoter, this step involves elevating the temperature of the cell (or the tissue or tumor it is contained in) to a temperature suitable for inducing the promoter. This requires elevating the temperature to above normal body temperature.

[0072] For example, in procedures involving human cells, the temperature of the cell and/or tissue is elevated to a temperature above normal human body temperature, 37°C. The temperatures and ranges discussed above are for human hosts, but it will be readily recognized that appropriate temperatures can be induced in other animals by determining the normal body temperature of the animal and elevating the temperature of the tissue to a temperature above the normal body

temperature. The elevated temperature need only be suitable for the heat shock promoter utilized in the vector.

[0073] The elevating of the temperature of the cell can be referred to as inducing hyperthermia. The use of hyperthermia with tumors has some beneficial affects (see, Dewey, W. and Freeman, M., "Rationale for Use of Hyperthermia in Cancer Therapy," pp. 372-378) and thus, the use of heat shock promoters has benefits that may be two-fold: regulation of expression of vector components, and hyperthermic benefits to tumor therapy.

[0074] While hyperthermia induces the heat shock promoters, it may not be desirable to increase the temperature of other tissues of the animal undergoing treatment. Therefore, the hyperthermia is preferably localized to the cell and/or tissue of interest. Devices and methods of accomplishing localized hyperthermia are known (see, for example, United States Patent No. 6,176,857 to Ashley for a METHOD AND APPARATUS FOR APPLYING THERMAL ENERGY TO TISSUE ASYMMETRICALLY) and can be used to accomplish the desired effect. Alternatively, hyperthermia can be induced in the general anatomical area that contains the tissue of interest.

[0075] The effect of the hyperthermia is likely to be greatest when the elevated temperature is maintained in the tissue for a duration of time. Therefore, it is preferred that the elevated temperature be maintained in the tissue of interest for a set period of time.

[0076] The increase in the temperature of the cell and/or tissue of interest will depend upon several factors, including the type of procedure being conducted. Preferably, the temperature of the tissue of interest is elevated to between

approximately 38° and 45°C. For procedures involving a vector utilizing the human hsp70B promoter, it is preferred that the temperature of the tissue is elevated to approximately 41°C.

[0077] The length of time during which the temperature of the cell and/or tissue of interest remains elevated will also depend upon several factors. Preferably, for procedures in which is desirable to enhance the diffusion of the vector through the tissue of interest, such as a solid tumor, the elevated temperature is maintained until the vector diffuses through the tissue. That is, it is preferable that the elevated temperature be maintained in the tissue of interest until the vector diffuses throughout the tissue of interest. Appropriate times can be determined for specific vector and tissue combinations. The time can be determined for the vector being utilized as appropriate by standard methods. Preferably, the elevated temperature is maintained in the tissue of interest for between approximately one and 72 hours. The duration of maintaining the elevated temperature may be brief, extended, or even intermittent in nature. The preferred duration of hyperthermia will depend on several factors, and should be optimized accordingly. Appropriate end points for hyperthermia include completion of diffusion of the vector through the tissue of interest.

[0078] Inducing hyperthermia in accordance with the methods of the present invention can be accomplished in a variety of manners. Essentially any technique that produces an appropriate increase in temperature in the cell and/or tissue of interest can be used. Preferably, techniques of raising temperature in tissue that allow for maintaining the elevated temperature over a period of time are used.

[0079] Several methods of inducing hyperthermia in tissue have been described. United States Patent No. 6,167,313 to Gray, et al. provides an overview of several techniques and methods. Any standard technique can be used to accomplish the desired hyperthermia. For example, an ultrasonic transducer can be employed to deliver a localized increase in tissue temperature. For an example of methods and apparatuses in accordance with this category, see United States Patent No. 5,620,479 to Diedrich. Alternatively, a technique commonly referred to as interstitial hyperthermia can be employed. Other alternative methods of inducing hyperthermia include exposing the tissue to microwave radiation (for example, see United States Patent No. 5,861,021 to Thome et al. and No. 5,922,013 to Fallick) or magnetic induction (see the '313 patent).

[0080] The method employed to induce hyperthermia can be optimized based upon the nature of the tissue of interest. For example, for deep tissues, such as a tumor in prostate tissue, interstitial hyperthermia will likely offer a better ability to control the hyperthermia. For surface tissues, a simple device, such as an ultrasonic transducer, will be sufficient.

[0081] The references cited in this disclosure, except to the extent they may contradict any statements or definitions made herein, are each hereby incorporated by reference in their entirety.

[0082] The foregoing disclosure includes the best mode devised by the inventor for practicing the invention. It is apparent, however, that several variations in accordance with the present invention may be conceivable to one of ordinary skill in the relevant art. Inasmuch as the foregoing disclosure is intended to enable such person to practice the instant invention, it should not be construed to be limited

thereby, but should be construed to include such aforementioned variations, and should be limited only by the spirit and scope of the following claims: